Walking down the "IL": The Newfound Marriage between IL-36 and Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is characterized by elevated concentrations of inflammatory cytokines and chronic lung inflammation (1). Tobacco use is a primary cause of COPD (2), though mechanisms leading to disease development remain unknown. Although management of COPD is possible, most patients will experience at least one exacerbation each year, representing a large burden on the healthcare system (2). Therefore, studies into mechanisms leading to COPD could highlight targets for immunotherapeutic development, helping to decrease the burden of this disease. Cigarette smoke has been shown to elicit the production of several inflammatory cytokines in bronchial epithelial cells, including the understudied IL-36 family, implicating these cytokines as potential players in smoking-induced chronic inflammation that could lead to COPD (3). To explore this possibility, in this issue of the *Journal*, Kovach and colleagues (pp. 173–182) report on their investigations into the relationship between IL-36 cytokine expression and lung inflammation in long-term smokers with and without COPD (4).

Discovered two decades ago, the IL-36 cytokines belong to the IL-1 superfamily and consist of three agonists—IL-36α, IL-36β, and IL-36γ—and the IL-36 receptor antagonist (IL-36Ra) (5). IL-36 agonists promote inflammation via IL-36R signaling (6), and they function primarily at barrier sites, including the skin, lung, and gut, where they can sufficiently initiate immune protection mechanisms against environmental challenges (7). Signaling through IL-36R results in MyD88-dependent activation of proinflammatory pathways leading to recruitment and activation of immune cells as well as antimicrobial activity (8). Importantly, IL-36 agonists also stimulate T cells and dendritic cells in the skin (9) and induce T-cell proliferation and polarization (10), suggesting that these cytokines serve as a bridge between the innate and adaptive immune systems. The inflammatory signals induced by IL-36 agonists are regulated by IL-36Ra, helping to maintain tissue homeostasis (11, 12). However, disruption of this balance is a hallmark of several inflammatory diseases, including psoriasis, inflammatory bowel disease, and arthritis (13). Given the role of IL-36 in inflammatory diseases, it is reasonable to expect that the dysregulation of IL-36 signaling could significantly contribute to smoking-induced COPD progression.

Although studies have shown that both IL-36α and IL-36γ exert proinflammatory effects in the lung (14–16) and are elevated upon infection with viruses or bacteria *in vivo* (17, 18), little is known about the role of IL-36 in long-term inflammation in the lung. Using cells isolated from wild-type mouse lungs, Kovach and colleagues first determined cell-specific production of IL-36 agonists. After stimulation with heat-killed *Klebsiella pneumoniae*, differential expression of IL-36 was observed, in which fibroblasts and macrophages displayed increased IL-36γ expression and type II alveolar epithelial cells displayed increased IL-36α expression. To determine whether cigarette smoke could induce IL-36 agonist expression in human lung cells, primary bronchial epithelial cells from nonsmokers were treated with cigarette smoke components for up to 7 days. Interestingly, temporal differences in IL-36 agonist expression were observed, such that IL-36γ was upregulated early before declining in expression, whereas IL-36α was upregulated later at Day 7. These results suggest a role for IL-36γ in mediating an early innate immune response whereas IL-36α may contribute more to chronic inflammation in the context of cigarette smoke component stimulation. Knowing that cells in both mouse and human lungs are responsive to elements leading to COPD, the authors next investigated patient plasma and BAL fluid (BALF) samples collected from long-term smokers (LTS) with or without COPD. Generally, systemic IL-36α and IL-36γ expression was found to be higher in LTS with or without COPD compared with nonsmoker control subjects. The exception to this was that little difference was observed between concentrations of IL-36γ in plasma from LTS with COPD compared with nonsmokers, which could support the idea that IL-36γ is elevated early in the immune response, serving a protective role. In addition, both IL-36α and IL-36γ were elevated locally in LTS with and without COPD compared with nonsmokers, with a trend toward higher concentrations of IL-36 agonists in BALF from patients with COPD compared with BALF from patients without COPD, though this was not statistically significant. An intriguing discovery came upon the observation that IL-36α protein concentrations in BALF were found to correlate with declining lung function as measured by forced expiratory volume in 1 second and airway obstruction as measured by the forced expiratory volume in 1 second/forced vital capacity ratio, suggesting that IL-36α could be a potential diagnostic marker for worsening lung function in COPD. To further investigate IL-36 agonists in the inflammatory cytokine milieu, correlations between a panel of Th1 and Th17 cytokines and IL-36γ and IL-36α were examined in BALF and plasma samples. Several positive correlations were found both locally and systemically, supporting the notion that elevated IL-36 agonist concentrations are associated with an elevated inflammatory response. Further investigation of immune cell responses to direct stimulation with IL-36 agonists using

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pulmonary macrophages demonstrated increased mRNA expression of TNFα and CXC and CC chemokines, which occurred in a MyD88- and IL-36R–dependent fashion. This held true as well for pulmonary macrophage stimulation with macrophage-derived microparticles from wild-type mice, as IL-36 cytokines are believed to be delivered in microparticles and exosomes (19). Conversely, microparticles derived from IL-36$\gamma^{-/-}$ mice or antibody-mediated inhibition of IL-36γ signaling resulted in no enhanced expression of measured chemokines and cytokines, suggesting that this stimulation was IL-36 agonist dependent.

Importantly, the authors expand on the limited available data surrounding contributions of IL-36 cytokines to inflammatory pathogeneses, providing a preliminary snapshot of spatiotemporal regulation of IL-36 signaling in the lung. This study has identified IL-36 as a clear marker of chronic lung inflammation in LTS and suggests a significant role for these cytokines in the progression of declining lung function. In addition, this work presents the potential for using IL-36 as a diagnostic tool in assessing lung damage in COPD. As this is a pilot study, additional investigation is required to tease out differences in IL-36–driven inflammation between LTS with and without COPD. In addition, further examination of specific mechanisms of IL-36–mediated inflammation are needed. Especially interesting is the interplay between IL-36α and IL-36γ in driving early and late stages of disease progression and determining the cause of the imbalance between agonist and antagonist. Overall, this study sets up the importance of investigations into the mechanisms of IL-36–induced inflammation in the lung. Understanding these pathways will aid in the development of targeted immunotherapies for treating COPD and related diseases, and this study brings the field one step closer to achieving this aim. ■

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